Triple oxygen isotope analysis of nitrate using isotope exchange - cavity ringdown laser spectroscopy

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Abstract

RATIONALE: Triple oxygen isotopes ($^{16}O/^{17}O/^{18}O$) in nitrate are a valuable tool to ascertain the pathways of nitrate formation in the atmosphere and the fate of nitrate in ecosystems. Here we present a new method for determining $\Delta^{17}O$ values in nitrates, based on nitrate-water isotope equilibration (IE) and subsequent isotopic analysis of water using cavity ringdown laser spectroscopy (CRDS).

METHODS: Nitrate oxygen ($O\text{-}NO_3^-$) is equilibrated with water oxygen ($O\text{-}H_2O$) at low pH and 80°C. Subsequently, the $\delta^{17}O$ and $\delta^{18}O$ values of equilibrated water are determined by CRDS, scaled to V-SMOW and V-SLAP and calibrated against nitrate standards (USGS-34, USGS-35 and IAEA-NO3). We provide isotopic measurements of synthetic and natural nitrates and a direct inter-lab with the classic method of thermal-decomposition of nitrate followed by isotope ratio mass spectrometry of O_2 (TD-IRMS).

RESULTS: For synthetic $NaNO_3$, the precision (1SD) of the IE-CRDS method is 0.8‰ for $\delta^{17}O$ values, 1.7‰ for $\delta^{18}O$ values and 0.2‰ for $\Delta^{17}O$ values when using a $O\text{-}NO_3^-/O\text{-}H_2O$ greater than 0.0114±0.0001 (e.g. 12 μmol of NO_3^- in 50 μL of acid

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solution). For natural samples, after purification of nitrates by column chemistry and reprecipitation as $AgNO_3$, the precision is better than 1.8‰ for $\delta^{17}O$ values, 3.2‰ for $\delta^{18}O$ values and 1‰ for $\Delta^{17}O$ values. IE-CRDS and TD-IRMS yield $\Delta^{17}O$ values within the analytical errors of the two methods.

CONCLUSIONS: The IE-CRDS method for determining $\Delta^{17}O$ values in nitrates utilizes a user-friendly and relatively cheaper benchtop analytical instrument, representing an alternative to IRMS-based methods for certain applications.

1. INTRODUCTION

Triple oxygen isotope measurements ($^{16}O/^{17}O/^{18}O$) have become an important part of the geochemical toolkit over the past two decades, influencing fields such as atmospheric chemistry[1-4], meteorites and planetary science[5-7], hydrology[8,9], paleoclimatology[10-12], atmospheric evolution[13-14], forensic studies[15,16], among other promising applications[17,18]. Many of these investigations focused on oxygen-bearing compounds in sediments and soils, in order to constrain the atmospheric processes and environmental conditions that prevailed at the time of mineral precipitation[3,11,19,20]. This is because, under certain conditions, some minerals (e.g. nitrates, sulfates and carbonates) record and preserve the isotopic composition generated in other oxygen reservoirs, including the atmosphere[4] and the hydrosphere[3,11,21]. In particular, triple oxygen isotopes in atmospheric nitrate particles[2], soil nitrate[3], nitrate in ice[22] and nitrate dissolved in natural waters[23-26] have provided key insights into the pathways of nitrate formation in the atmosphere and the fate of nitrate in ecosystems.

The $^{18}O/^{17}O/^{16}O$ anomalies ($\Delta^{17}O$ values) in oxygen-bearing compounds can be expressed as deviations with respect to the oxygen isotope ratios in Earth natural waters[17,18]. In the present study, we use the following definition for $\Delta^{17}O$:

$$\Delta^{17}O = \ln(\delta^{17}O+1) - 0.528 \ln(\delta^{18}O+1) \quad \text{(Equation 1)}$$

where the $\delta^{17}O$ and $\delta^{18}O$ value denote the $^{17}O/^{16}O$ and $^{18}O/^{16}O$ ratios in water standardized to V-SMOW (Vienna Sea Mean Ocean Water) and SLAP (Standard Light Antarctic Precipitation)[8, 27,28]. The value of 0.528 has been proposed to

describe the $\delta^{17}O$ and $\delta^{18}O$ relationship in rainwater worldwide[8], and we use equation 1, given that we primarily analyze water samples in this study. Note that $\Delta^{17}O$ (usually specified in parts per thousand or ‰ units) is equivalent to $^{17}O_{excess}$ (usually specified in parts per million or "per meg" units), which is used extensively in investigations on triple oxygen isotopes in the hydrologic cycle[8,9,11]. In this study, we use $\Delta^{17}O$ when referring to large triple oxygen isotope anomalies, such as those observed in solids (e.g. nitrates) and ^{17}O-spiked waters, whereas $^{17}O_{excess}$ is used when discussing natural waters and isotopically equilibrated samples (normally <<1000 per meg or 1‰). Note that some previous studies used alternative definitions for triple oxygen isotope anomalies, which yield slightly different values of $\Delta^{17}O$ for equivalent $\delta^{17}O$ and $\delta^{18}O$ values[17, 29]; thus, caution must be taken when comparing directly $\Delta^{17}O$ values from different studies.

Nitrates from atmospheric deposition generally show large $\delta^{18}O$ values[30] (e.g. 40‰ to 80‰) with extremely large triple oxygen isotope anomalies[30] ($\Delta^{17}O$ from 15‰ to 33‰). This is because of photochemical reactions of NO_x with atmospheric O_3, which carries the primary "anomalous" oxygen isotope signature from mass-independent fractionation (O-MIF) processes during stratospheric ozone formation[31]. Reactions with O_3 also transmit $\Delta^{17}O$ values to other atmospheric species which can transfer to the surface in wet or dry deposition (e.g. sulfates and perchlorates, etc.), but nitrate aerosols retain (and preserve) the largest known natural solid-phase $\Delta^{17}O$ values.

The first measurements of triple oxygen isotopes in nitrate were performed by Michalski et al [32], whose method consists of determining the $\delta^{18}O$ and $\delta^{17}O$ values in nitrate by generating O_2 through the thermal decomposition of $AgNO_3$, followed by isotopic analysis of the O_2 by Isotope Ratio Mass Spectrometry (TD-IRMS) (Table 1). Later, Kaisier et al[33] developed a method based on the conversion of nitrate to N_2O using a bacterial denitrifier strain and subsequent decomposition of N_2O to N_2 and O_2 in a gold furnace at 800°C, followed by gas chromatographic separation and isotope analysis of O_2 by IRMS. This procedure requires considerably less nitrate (~50 nmol) than the Michalski et al method (6-40 μmol) but maintains comparable $\Delta^{17}O$ precision (0.5‰). This bacterial denitrification method was later automated by Morin et al[34] and modified to permit measurements of $\delta^{15}N$ values together with triple

oxygen isotopes in the same nitrate sample (Table 1). Likewise, Kunasek et al[35] modified the TD-IRMS method by conducting the nitrate decomposition in a high temperature conversion elemental analyzer (TC/EA) at 550^{o}C. The main difference between the later method and that described in Michalski et al[32] is the use of a continuous flow of helium in the on-line pyrolysis system to carry sample to the mass spectrometer, whereas the original procedure utilized an off-line preparation of O_2 under vacuum. Schauer et al[36] reported that $\Delta^{17}O$ results obtained by TD-IRMS method can be compromised due to oxygen isotope exchange between O_2 and quartz at high temperatures when quartz cups are used to introduce the samples in the TC/EA furnace, and recommended silver capsules for the TD-IRMS method. The updated TD-IRMS methods for $\Delta^{17}O$ in nitrates show reproducibility in the range of 0.2‰ to 0.4‰ (1SD) (Table 1).

Alternatively, Komatsu et al[37] used a chemical procedure involving cadmium sponge and $NaHCO_3$ solution to convert dissolved nitrate to N_2O[38,39], followed by direct $\Delta^{17}O$ determination in N_2O by IRMS, with reproducibility of 0.2‰. More recently, Dyckmans et al[40] developed a method based on the conversion of nitrate to N_2O using bacteria, similar to that of Kaiser et al[33], but in this case decomposition of N_2O to N_2 and O_2 is performed by a microwave-induced plasma in a corundum tube. An automatized version of the Dyckmans et al[40] method was developed by Hattori et al[41], with overall external $\Delta^{17}O$ precision of 0.3‰. Finally, Wassemaan et al[42] developed an innovative method based on dissolved NO_3^- reaction with Cd-azide and analysis of the evolved N_2O by laser spectroscopy (Table 1).

Although relatively high $\Delta^{17}O$ precisions can be achieved by these methods (~0.1 to 0.5‰), several significant inconveniences have stopped the implementation of some of these analytical methods on a wider basis. These include the use of costly setups (e.g. gold furnace)[33] and hazardous reagents (e.g. Cd-azide)[42], as well as extremely expensive analytical instruments (i.e. isotope ratio mass spectrometers).

New analytical techniques, including Cavity Ringdown laser Spectroscopy (CRDS) and Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS), which are less expensive and more user-friendly than mass spectrometry, have permitted high precision measurements of triple oxygen isotopes in liquid waters[43-45], hydration water in minerals[11,19,46], water fluid inclusions in speleothems[47] and gaseous nitrogen

oxide[42]. It is only very recently, however, that methods for triple oxygen isotopes in solid materials and dissolved salts using laser spectroscopy-based analyzers have been presented[42,48].

Laboratory experiments have demonstrated that oxygen isotopes in nitrate ($O\text{-}NO_3^-$) exchange and equilibrate with water oxygen isotopes ($O\text{-}H_2O$) at low pH (<1.1) and temperatures above 50°C; while no isotopic exchange is observed at lower temperature and circumneutral pH[49]. This means that (1) nitrate can preserve its primary oxygen isotopic composition after precipitation if not exposed to extreme conditions and (2) isotopic equilibration with water is possible under acid wet laboratory conditions.

Because oxygen equilibration involves an isotope mass-balance, any observed change in the $\delta^{18}O$ value of $O\text{-}NO_3^-$ after equilibration is accompanied by an equivalent change in the $\delta^{18}O$ value of $O\text{-}H_2O$. As suggested by Kaneko and Poulson[49], the $O\text{-}NO_3^-/O\text{-}H_2O$ isotopic equilibration is expected to follow a mass-dependent law for triple oxygen isotopes[50] (i.e. $\delta^{17}O=\delta^{18}O^{\theta}$, where θ~0.52). Hence, provided adequate proton concentration and heat, the oxygen isotope composition of nitrates, including ^{17}O anomalies, will be "transmitted" to water and vice-versa. The isotopic composition ($\delta^{17}O$, $\delta^{18}O$ and $^{17}O_{excess}$ values) of the equilibrated water can then be measured with high precision (better than 15 per meg) by CRDS[44]. By following this approach, we have developed a new method to determine $\Delta^{17}O$ in nitrate based on $O\text{-}NO_3^-/O\text{-}H_2O$ isotope equilibration, followed by isotope analysis of water by CRDS.

2. EXPERIMENTAL AND ANALYTICAL METHODS

2.1. Preparation of internal nitrate standards

Internal nitrate standards ($NaNO_3$) with different $\delta^{17}O$, $\delta^{18}O$ and $\Delta^{17}O$ values were generated by the equilibration method described by Böhlke et al.[51] The oxygen isotopes of HNO_3 (~65% by weight, VWR Chemicals Prolab, Lutterworth, UK; no. 20429.320) were equilibrated with oxygen in DI water (deionized water with electrical conductivity <15 µS/m) spiked with different amounts of ^{17}O-enriched water (10%, Berry & Associates, Dexter, MI, USA). Four simultaneous equilibration procedures were conducted to create internal $NaNO_3$ standards, described here as ST-01, ST-02, ST-05 and ST-06. The measured $\Delta^{17}O$ values of our mixed ^{17}O-enriched waters ranged from 2.2‰ to 54.0‰. The initial isotopic composition of the total solution in our equilibrations will differ from that measured in the ^{17}O-enriched waters, as the isotopic composition of the water in the bulk HNO_3 acid (~35% H_2O) is unknown. The ^{17}O-enriched H_2O/HNO_3 solutions (1M, pH=0) were equilibrated in 500-mL tightly capped Nalgene HDPE plastic bottles at 80±0.1°C in a Memmert™ Natural Convection Oven (Fisher Scientific, Loughborough, UK) for 7 days. Afterwards, the solutions were rapidly introduced into an ice-water bath to "quench" the O-isotope exchange between NO_3^- and H_2O. The acid solutions were neutralized by slowly adding 10M NaOH (+99.5%, Fisher Scientific; no. S/4920/53), also with unknown oxygen isotope composition. During neutralization, the solutions were immersed in an ice-water bath to dissipate heat released from the reaction. The pH of the solutions was monitored until final pH values ranging from 6.5 to 7.5 were achieved. These neutral solutions were air-dried for 3-4 days at 35°C. The precipitated $NaNO_3$ was then ground to a fine powder using an agate mortar to remove fluid inclusions in crystals and further homogenize the material. The ground $NaNO_3$ was dried for 1 day at 35°C and finally stored in amber glass bottles. Our four synthetic $NaNO_3$ standards with different O-MIF values, along with an additional in-house standard of analytical grade $NaNO_3$ (namely ST-03; +99%, Acros Organics, via Fisher Scientific; no. 205960010) with expected $\Delta^{17}O \approx 0$, were analyzed for their $\delta^{17}O$, $\delta^{18}O$ and $\Delta^{17}O$ values and calibrated using the methods described below.

2.2. O-NO_3^-/O-H_2O isotope equilibration (IE) and subsequent isotopic analysis of water using cavity ringdown laser spectroscopy (CRDS)

2.2.1. General O-NO_3^-/O-H_2O isotope equilibration method

Equilibration experiments were performed in Hungate type anaerobic culture tubes, 16x125mm, with Butyl stoppers and screwed cap (Fisher Scientific; no. 501215187). Nitrate salts were weighed to a precision of ±0.1 mg. Subsequently, 500 μL of 0.79M HCl (pH=0.1; referred as ST-HCl hereafter), sourced from analytical grade HCl (12N; Fisher Scientific) and diluted with DI water with known and constant oxygen isotopic composition (referred as ST-H_2O hereafter), was added to the tube and weighed again. In our experiments, the O-NO_3^-/O-H_2O molar ratio (called β hereafter) ranged from 0.0011 to 0.0581 (e.g. 12 to 590 μmol of NO_3^- in 500 μL of ST-HCl, or 6 to 295 μmol in 250 μL), with a precision of ±0.0001 (1SD). The tubes were capped and placed in the oven at 80°C±0.1°C, typically for 3 to 5 days. Subsequently, the tubes were rapidly introduced in an ice-water bath to "quench" the O-isotope exchange between NO_3^- and H_2O. After ~30 min, the tubes were vigorously shaken. Because the analysis of acid solution can pose a serious threat for the Picarro (Santa Clara, CA, USA) vaporizer and CRDS analyzer (e.g. deterioration of valves and potential damage of the cavity's mirrors), solutions were neutralized before the oxygen isotopic analysis. To this end, 41.5 μL of 10M NaOH, created by dissolving NaOH (+99.5%, Fisher Scientific; S/4920/53) in ST-H_2O (referred as ST-NaOH hereafter), was added to neutralize the 500-μL acid solution, then capped and shaken. Note that the NaOH is kept at 4°C before use, in order to aid dissipation of heat released from the neutralization reaction. Once the pH was neutral, the solutions were stored at room temperature until analysis. 250 μL of solution was transferred into 2-mL septum-capped glass vials, fitted with a 300-μL micro-insert (Fisher Scientific; no. 10376102), and then loaded into a Picarro A0325 autosampler.

Two types of blank experiments were performed together with the equilibration experiments: (1) blanks consisting of 500 μL of ST-H_2O and (2) blanks consisting of 500 μL of ST-HCl that were neutralized in the same way as the equilibration experiments. The former accounts for any potential water evaporation during the time of the experiments in the oven and isotopic fractionation during sample

manipulation (e.g. pipetting). The later neutralized blank similarly accounts for evaporation but allows evaluation of three sources with unknown oxygen isotope compositions. This includes (i) water from the original concentrated HCl that represents ~4% of the initial solution in the equilibration experiments; (ii) water from the ST-NaOH solution, representing <0.01% of the solution, whose isotopic composition of the original ST-H_2O may have been modified because of equilibration with OH^- (aq) from the NaOH with unknown isotopic composition; (iii) water generated from the neutralization reaction and in which the oxygen also derives from OH^- (aq), representing ~0.8% of the final solution; and (iv) presumed isotope exchange with the borosilicate glass of the tubes at high temperature and low pH. In summary, the ST-HCl blanks account for presumed water evaporation, isotopic fractionation during sample handling and any isotopic background produced by the ~5% of water with unknown isotopic composition derived from the reagents in the experimental protocol.

2.2.2. O-NO_3^-/O-H_2O isotope equilibration of small samples

Sample amount can be a limiting factor for some applications of our method (e.g. nitrates in ice and freshwaters; see section 3.4), so we explored reducing the solution volume in the O-NO_3^-/O-H_2O equilibration step in order to decrease the sample size required. To this end, 9 μmol of NO_3^- (±0.1 μmol) (e.g. 800 μg of $NaNO_3$ salt) were dissolved in 50 μL of ST-HCl solution (i.e. β = 0.0091±0.0001) into disposable 6-mm break-seal borosilicate tubes (Fisher Scientific; no. 0674620). The sample tubes were immediately connected to a vacuum line composed of ¼-inch O.D. stainless steel tubing with an I.D. of ~4 mm, attached to a 1-m flexible stainless-steel pipeline; an Ultra-torr union fitted with a VitonTM O-ring and a stainless-steel Ball Valve, 1/4 in. (Swagelok, Aberdeen, UK). A Pirani gauge (APG100 Active Pirani vacuum gauge of Edwards, Bolton, UK) is fitted to the line for monitoring pressure via an Edwards TIC head instrument controller. The line was connected to a two-stage rotary pump (Edwards E2M2) and the flexible stainless-steel pipeline immersed in liquid nitrogen to improve vacuum efficiency. Before placing the system under vacuum, the water sample was frozen into the base of the break-seal tube by immersion in liquid nitrogen to avoid water evaporation. Afterwards, the non-condensable gases were pumped away for 30 seconds to a pressure of 10^{-2} mbar.

Finally, the 6-mm break-seal tubes were flame sealed and placed in the oven at 80^{o}C for at least three days.

After equilibration (same conditions as section 2.2.1), the tubes were rapidly placed in an ice bath. Prior to analysis, water was frozen into the base of the break-seal tube by immersion in liquid nitrogen. Subsequently, the tube was scored with a diamond cutter, broken to a fixed height of 25 mm, and then quickly inserted into the 2-mL septum-capped vials used by the Picarro A0325 autosampler[45]. Prior to the analysis, the solution was neutralized by adding 4.2 μL of the ST-NaOH solution (10N).

2.2.3. Equilibration time and sample amount sensitivity

To evaluate the time required by the O-NO_3^-/O-H_2O system to achieve isotopic equilibrium, we carried out equilibration experiments using nitrate standards, ST-01 and ST-03. The experiments consisted of 234±1 μmol of NO_3^- (i.e. 20 mg of $NaNO_3$) dissolved in 500 μL of ST-HCl solution and equilibrated at 80^{o}C for different periods of time (1 h, 3 h, 6 h, 16 h, 24 h, 46 h, 72 h, 96 h and 528 h). The experiments were performed in duplicate using the methods described in section 2.2.1, except for the equilibration time.

The sealed tube ensures isotopic mass-balance in the O-NO_3^-/O-H_2O equilibration reaction, so the oxygen isotope composition of the species in the equilibrium depends solely on the molar ratio between nitrate and water, their initial isotopic compositions, and the equilibrium isotope fractionation factor (α) of the reaction. We assume that the equilibrium isotope fractionation factor (α) remains a constant under identical experimental conditions, so conduct all experiments at the same pH and temperature. To further evaluate the sensitivity of the isotopic composition of the water after various times of equilibration, we explored differing β values, utilizing different amounts (12, 35, 71, 119, 176, 235, 355 and 590 μmol) of nitrate from internal standards ST-01, ST-02, ST-04, ST-05 and ST-06 equilibrated with 500 μL of ST-HCl solution at 80^{o}C. The experiments were performed in duplicate using the methods described in section 2.2.1, except for the sample amount.

2.2.4. Calibrations with international reference nitrates

A typical run of equilibration experiments consists of 10-12 unknowns (4-5 duplicated samples), 3 standards run in duplicate, 1 ST-H_2O blank and 1 ST-HCl blank. The same molar amount of NO_3^- (±0.1 μmol) was used for unknown and standards in each set of experiments. Our three-point calibration line using the international reference materials covers the oxygen isotopic composition of most of the samples analyzed in this study and most nitrates observed in nature. We run our 5 synthetic nitrates (ST-01, ST-02, ST-03, ST-05 and ST-06) together with the international standards USGS-34 ($\delta^{18}O$=-27.9‰ and $\Delta^{17}O$=0‰), USGS-35 ($\delta^{18}O$=+57.5‰ and $\Delta^{17}O$=+20.7‰) (both from National Institute of Standards and Technology, Gaithersburg, MD, USA) and IAEA-NO3 ($\delta^{18}O$=+25.6‰ and $\Delta^{17}O$=-0.2‰) (International Atomic Energy Agency, Vienna, Austria). Standards USGS-34 and IAEA-NO3 are KNO_3, while USGS-35 and our internal synthetic standards are $NaNO_3$, so care was taken to ensure that equivalent amounts of NO_3^- were used. To evaluate the sensitivity of the calibration of unknowns to sample size we conducted calibrations with different nitrate amounts (i.e. β from 0.0011 to 0.0581).

2.2.5. Natural samples

One soil sample (sample AT12 PONR-90, taken at 90 cm depth from a soil profile at 23°04'22,0''S; 69°35'21,9''W) and two microcrystalline aggregate mineral samples (samples S-2 and S-6, 24°05'17.9''S; 69°59'40.9''W), all of them containing high nitrate concentrations, were collected from different parts of the hyper-arid Atacama Desert (Chile) and analyzed here by the IE-CRDS method (Table 2). The results were calibrated against ST-01, ST-02 and ST-03, and IAEA-NO3 was analyzed as an unknown used to evaluate accuracy. Prior to the analysis, the salt mixtures samples were gently ground to a fine powder and dried in the oven at 50°C overnight. Subsequently, the powders (~50 mg) were poured into 50 mL of deionized water, then kept sealed overnight at 50°C, centrifuged, and passed through a 40-micron filter. Soil samples (1 g) were poured into 50 mL of DI water and kept at 30°C overnight, following by centrifugation and filtering.

10 mL of $BaCl_2$ solution (50 g/L) was added to each 50 mL of solution to precipitate any sulfates and phosphates. The tubes were centrifuged, and the supernatant was transferred to new tubes. In addition to sulfates, a complex mixture of other salts (e.g., chlorides and perchlorates) in solution is expected, so dissolved nitrates were purified and converted to $AgNO_3$ using anion exchange resin AG1-X8 (200-400 mesh in the chloride form, Bio-Rad, Hercules, CA, USA) using the method described by Silva et al[52]. The internal standards ST-01, ST-02 and ST-03 (previously calibrated against USGS-34, USGS-35 and IAEA-NO3, see previous section) were treated in the same way and converted to $AgNO_3$. The resulting $AgNO_3$ was analyzed using the procedure described in section 2.2.1. To this end, 14 mg of $AgNO_3$ (82 μmol of NO_3^-) was dissolved in 250 μL of ST-HCl solution (i.e. β=0.0162±0.0001). $AgNO_3$ reacts with HCl to produce insoluble AgCl and HNO_3. The $AgNO_3$ from natural samples was also independently analyzed for $\delta^{18}O$ values using the method to be described in section 2.4.

2.2.6. Isotopic analysis of water by CRDS

Isotope analyses were conducted at the School of Earth and Environmental Sciences, University of St. Andrews (St Andrews, UK). Water oxygen and hydrogen isotopes were measured simultaneously by CRDS using a L2140-i Picarro water isotope analyser[44], interfaced with an A0211 high-precision vaporizer. The instrument used N_2 gas as carrier. Because of the relatively high salinity of the solutions analyzed in this study (up to ~100 g/L), long-term accumulation of salts in the vaporizer can pose an issue for routine analyses. We use a metal mesh insert that is introduced into the vaporizer port and filled with glass wool to retain salts and prevent them from accumulating in the vaporizer. The insert was replaced and cleaned at the beginning of every run.

Each sample was injected ten times into the vaporizer, which was heated to 110°C. Memory effects from previous samples were avoided by rejecting the first three analyses. Values for the final 7 injections were averaged with a typical mean instrumental precision (±1SD) of ±0.02‰ for $\delta^{17}O$ values, ±0.03‰ for $\delta^{18}O$ values and ±0.19‰ for $\delta^{2}H$ values, as observed from repeated analysis of an in-house water standard (n=127) over one year. The results were normalized against V-SMOW by analyzing internal standards before and after each set of fifteen to twenty

samples. To this end, three internal water standards (JRW, BOTTY, SPIT) were calibrated against V-SMOW and SLAP, using $\delta^{17}O$ values of 0.0‰ and -29.69865‰, respectively, and $\delta^{18}O$ values of 0.0‰ and -55.5‰, respectively[29]. This approach assumes that V-SMOW and SLAP have $^{17}O_{excess}$=0. All the $^{17}O_{excess}$ values are given in per meg units (0.001‰). The in-run drift of the instrument was monitored by analyzing a water standard every 6 samples. No drift was observed, so no correction was necessary. The $^{17}O_{excess}$ value was calculated for each injection using the normalized $\delta^{17}O$ and $\delta^{18}O$ values. The $^{17}O_{excess}$ values from the 7 repeated analyses were then averaged. Typical in-sample $^{17}O_{excess}$ and d-excess precisions (1SD) in water standards (BOTTY, n=127), were 13 per meg. No $^{17}O_{excess}$ values were rejected.

2.3. Triple oxygen isotope analyses by thermal decomposition followed by IRMS (TD-IRMS)

Synthetic nitrates (ST-01, ST-02, ST-03, ST-05 and ST-06) were also analyzed for $\Delta^{17}O$ by the classic method of thermal decomposition of nitrate followed by isotope analysis of O_2 by IRMS[33] at the Department of Geology and Geophysics, Louisiana State University (Baton Rouge, LA, USA. The $NaNO_3$ was converted to O_2 by heating the sample to 520^{o}C *in vacuo* (10^{-7} torr) in borosilicate tubes. After cryogenic trapping of evolved NOx, the evolved O_2 was cryogenically trapped in a cold finger using 5A molecular sieves at 77K. The triple oxygen isotope ratios of O_2 were determined using a Finnigan-Mat 251 isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) with Faraday cup detectors at *m/z* 32, 33, and 34. The $\Delta^{17}O$ values of the unknows are calibrated against the reference materials IAEA-NO3 and USGS-35. The typical $\Delta^{17}O$ precision of the method is ±0.2‰ (1SD).

2.4. Measurement of $\delta^{18}O$ values by High Temperature Conversion Elemental Analyzer coupled to IRMS (TC/EA-IRMS)

For $\delta^{18}O$ analysis of synthetic nitrates (ST-01, ST-02, ST-03, ST-05 and ST-06), a High Temperature Conversion Elemental Analyzer (TC/EA) (Thermo Scientific) was used to pyrolyze the nitrate at 1450ºC and produce CO, which was measured using a continuous flow Gas Source isotope-ratio mass spectrometer (Delta V Plus, Thermo Scientific) at the Department of Earth Sciences, University of Cambridge

(Cambridge, UK). Samples (~100 μg) were loaded in tin capsules and run four consecutive times, bracketed by the international nitrate standards, USGS-34, USGS-35 and IAEA-NO3. IAEA-NO3 was analyzed every 15 samples to monitor the drift of the instrument. The typical in-sample precision of the unknown and the standards was ±0.5‰ (1SD). All $\delta^{18}O$ values are relative to V-SMOW.

3. RESULTS AND DISCUSSION

3.1. Blanks and background

The IE-CRDS method described here uses blanks to evaluate the effect of a number of processes and sources of oxygen that may affect the magnitude of the results, including water evaporation and isotope fractionation when handling the solutions, isotopic exchange between the water and the borosilicate tubes and the contribution of waters with unknown isotope composition from the reagents (i.e. concentrated HCl and NaOH).

The isotopic composition of the ST-H2O standard before the experiments ($\delta^{17}O$= -4.13±0.03‰; $\delta^{18}O$= -7.88±0.05‰; $\delta^{2}H$ =-52.8±0.4‰; $^{17}O_{excess}$=35±11 per meg; n=21) is indistinguishable from that of the same water placed in the oven at 80^{o}C in the Hungate tubes for at least 3 days ($\delta^{17}O$= -4.14±0.05‰; $\delta^{18}O$= -7.88±0.09‰; $\delta^{2}H$ =-53.0±0.4‰; $^{17}O_{excess}$=35±13 per meg; n=23). This confirms that no evaporation occurs during the extent of the experiments, nor isotope fractionation during solution transferring, as higher $\delta^{17}O$, $\delta^{18}O$ and $\delta^{2}H$ values and lower $^{17}O_{excess}$ values, would be expected for evaporated waters[9,11,44].

The second type of blank in our method involves the acid solution ST-HCl. The isotopic composition of the ST-HCl blanks after the equilibration period and neutralization ($\delta^{17}O$= -4.19±0.04‰; $\delta^{18}O$= -7.98±0.08‰; $\delta^{2}H$=-55.4±0.9‰; $^{17}O_{excess}$=37±8 per meg; n=22) shows slightly more negative values of $\delta^{17}O$ and $\delta^{18}O$ (approximately 0.1‰ for $\delta^{18}O$ and 0.05‰ for $\delta^{17}O$) than the ST-H2O standard used to make the ST-HCl solution. Importantly, the $^{17}O_{excess}$ of the ST-HCl after the experiment is not altered. Although the $\delta^{2}H$ measurements are not relevant for our final application, the $\delta^{2}H$ value of the ST-HCl blank after equilibration/neutralization

was observed to be 2.5‰ lower than that of the ST-H2O standard. In summary, this indicates that waters with unknown isotope composition from the HCl concentrated solution, the ST-NaOH solution and/or the neutralization reaction significantly affect the δ^2H value, have an minor impact on the $\delta^{17}O$ and $\delta^{18}O$ values, and do not alter the $^{17}O_{excess}$ of the final solution. These combined results also rule out significant isotope exchange between water and the borosilicate at the experimental conditions (pH=0.1 and 80^{o}C). Given the above demonstrations, we assert that the IE-CRDS method for oxygen isotope measurements on nitrate does not require background/blank corrections provided that all samples and standards are processed identically.

3.2. Isotopic equilibration and sample size dependence

As expected, we found that the isotopic composition of the ST-HCl acid solution equilibrated with dissolved NO_3^- (constant β of 0.0232±0.0001) at 80°C changed with time, reaching a plateau after ~24h (Figure 1). This performance was slightly faster than in similar experiments reported by Kaneko and Poulson[49] in which 98% equilibration occurred after 121 h. In practice, we extend our experiments to at least 72 h. Longer equilibration periods do not influence the final isotope values.

The isotope equilibrium composition of water (e.g. the $\delta^{18}O\text{-}H_2O_{eq}$ value in Figure 1A) depends on the isotope composition of initial nitrate. For example, the $\delta^{18}O\text{-}H_2O_{eq}$ value of water in equilibrium with our ST-01 synthetic nitrate with initial $\delta^{18}O\text{-}NO_3^-$ =47.2±0.8‰, (measured by TC/EA-IRMS; Table 2) is -7.0‰, whereas it is -7.6‰ when the dissolved nitrate is ST-03 with initial $\delta^{18}O\text{-}NO_3^-$=26.3±0.7‰. Likewise, the $^{17}O_{excess}$ of water equilibrated with the ST-01 nitrate reaches a constant value after 24 h (Figure 1B), with a $^{17}O_{excess}$ of 600 per meg, ~570 per meg higher than that of the initial ST-HCl solution and considerably elevated compared with natural waters[8] (e.g. ~37 per meg). In contrast, the water equilibrated with the ST-03 standard shows constant $^{17}O_{excess}$ with time. This is because the ST-01 has a triple oxygen isotope

anomaly ($\Delta^{17}O$) of 23.2‰ (measured by TD-IRMS; Table 2), whereas the ST-03 nitrate is a commercial analytical reagent with no MIF-anomaly ($\Delta^{17}O$=-0.1‰).

We evaluated the change in $^{17}O_{excess}$ experienced by the ST-HCl solution after complete equilibration with different amounts of dissolved nitrate (i.e. β from 0 to 0.0581 at a fixed volume of 500 μL) with $\Delta^{17}O$ ranging from -0.1‰ to 45.4‰ (measured by TD-IRMS). The $^{17}O_{excess}$ of the equilibrated ST-HCl solution linearly increases with sample size (i.e. increasing β) with R^2 better than 0.97 in the experiments performed with O-MIF nitrates (ST-01, ST-02, ST-05 and ST-06) (Figure 2). The $^{17}O_{excess}$ of the exchanged water reaches values up to ~2800 per meg (2.8‰) for β of 0.0581 (e.g. 590 μmol of NO_3^- in 500 μL of acid solution). However, when the nitrate ST-03 ($\Delta^{17}O$=-0.1‰) is used, as expected for equilibration with O-MDF nitrate, the measured $^{17}O_{excess}$ barely changes with increasing amount of nitrate in solution.

Importantly, the slope of β vs$^{17}O_{excess}$-H_2O_{eq} in Figure 2 is directly proportional to the $\Delta^{17}O$ value of the O-NO_3^-. The lines fitted on Figure 2 represent the mixing path between O-H_2O and O-NO_3^-, where a β of 0 means that all the oxygen in the mixture corresponds to water (e.g. no nitrate in solution) and a β equal to 1 would imply (implausibly) that all the oxygen corresponds to nitrate. Note that β values greater than 0.8 are physically impeded by the solubility product of $NaNO_3$ (e.g. ~1400 g/L at 80°C) and technically impeded by the inability of the A0211 vaporizer to take samples with salinities higher than 100 g/L (β of 0.0581). If it is assumed that the O-NO_3^-/O-H_2O relationship remains linear over the range of β from 0.0581 to 1, the $^{17}O_{excess}$ (or $\Delta^{17}O$ value) of the O-NO_3^- end-member can be calculated from the linear regressions, when β is equal to 1. The corrected $\Delta^{17}O$ values of ST-01, ST-02, ST-03 and ST-05 calculated by this method are in perfect agreement with the results obtained by the classic TD-IRMS method, as shown in Table 2. The sole exception is for the extremely high $\Delta^{17}O$ value of 48.6‰ computed for the ST-06 standard, which is offset by ~3‰ with respect to the value determined by TD-IRMS (45.4‰). This disagreement could arise because the $\Delta^{17}O$ value of ST-06 is well beyond the calibration range of the TD-IRMS measurements, which were from 0 to 20.7‰.

From the results described above it can be concluded that: (1) the isotope exchange observed by Kaneko and Paulson[49] and measured in NO_3^- after water equilibration is also directly observable in the isotopic composition of the equilibrated waters; (2) the system reaches equilibrium conditions after 24h; (3) the $\delta^{18}O\text{-}H_2O_{eq}$ value is proportional to the isotopic composition of the initial nitrate; and finally, (4) the triple oxygen isotope composition of nitrate is transferred to water, confirming that the $O\text{-}NO_3^-/O\text{-}H_2O$ isotope equilibration reaction is a mass-dependent process governed by a θ~0.52.

3.3. Calibration of unknowns, precision, accuracy and inter-lab comparisons

To determine the triple oxygen isotope composition of unknown nitrate samples, we calibrate the $\delta^{17}O$, $\delta^{18}O$ and $^{17}O_{excess}$ values observed in the ST-HCl solution after equilibration with unknowns, against the change observed when international reference materials with known isotope compositions (USGS-34, USGS-35 and IAEA-NO3) are equilibrated under identical conditions and $O\text{-}NO_3^-/O\text{-}H_2O$ ratio (β). After equilibration and solution neutralization, the $\delta^{17}O$ and $\delta^{18}O$ values of $O\text{-}H_2O$ (e.g. the $\delta^{18}O\text{-}H_2O_{eq}$ value) increase when the most isotopically enriched reference material is used (USGS-35) and decrease when water is equilibrated with the nitrate with the lowest $\delta^{17}O$ and $\delta^{18}O$ values (USGS-34) (Figures 3A and B). As expected, equilibration with the large O-MIF USGS-34 nitrate increases the $^{17}O_{excess}$ of $O\text{-}H_2O$, whereas no measurable change in $^{17}O_{excess}$ was observed when equilibrating with the O-MDF standards. The extent of the change depends on the amount of nitrate in solution (β) (Figure 3C). The mean reproducibility (1SD) of the duplicated measurements was ±0.02‰ for $\delta^{17}O$ values, ±0.03‰ for $\delta^{18}O$ values and 7 per meg for $^{17}O_{excess}$, in good agreement with the internal reproducibility of the CRDS instrument. We found that the reproducibility of $O\text{-}H_2O_{eq}$ does not depend on β, suggesting that it is constant parameter derived from the CRDS analyzer performance and that our sample preparation method does not introduce any external variability.

There is a strong linear correlation between the $\delta^{17}O$-H_2O_{eq} and $\delta^{18}O$-H_2O_{eq} values and the known values of the reference materials (i.e. $R^2 > 0.95$ when $\beta \geq 0.007$) (Figure 3). This linear correlation is used to calculate the $\delta^{17}O$ and $\delta^{18}O$ values, and the derived $\Delta^{17}O$ values of the unknowns (referred to as δ-calibration hereafter). For β less than 0.007 (i.e. less than 72 μmol of NO_3^- in 500 μL of ST-HCl solution), the derived $\delta^{17}O$ and $\delta^{18}O$ values are inconsistent and highly imprecise. However, when β from 0.0117 to 0.058 is used (here 118 to 590 μmol of NO_3^- in 500 μL of ST-HCl solution) the isotopic compositions stabilize and 1SD better than 1.7‰ for $\delta^{18}O$ values can be obtained, increasing to 0.5‰ when β=0.058 (Fig. 4). The $\delta^{18}O$ values obtained from the IE-CRDS method are thus in good agreement with the results from the TC/EA-IRMS technique, within the analytical uncertainties of both methods (Figure 5).

Likewise, the precision of the $\Delta^{17}O$ results from our IE-CRDS method improves with sample size. Reproducibility better than 0.7‰ (1SD of duplicated analyses) can be achieved for $\beta \geq 0.007$ and the precision can be improved to 0.2‰ when $\beta \geq 0.0117$ is used, with no significant improvement above this value of β (Figure 4). The $\Delta^{17}O$ results of our synthetic nitrates by the IE-CRDS method agree with the $\Delta^{17}O$ values for the classic TD-IRMS technique (Figure 5B and Table 2), except for sample ST-06 for which the IE-CRDS result is ~4‰ higher. Again, this is probably because the $\Delta^{17}O$ values of ST-06 is far away from the calibration range of both methods, given that the isotopic compositions of the three international reference materials used span 0 to 20.7‰.

In addition to the δ-calibration, we test an alternative calibration method to determine the $\Delta^{17}O$ based on the determined $^{17}O_{excess}$ of water after equilibration (referred as Δ-calibration hereafter) instead of using both the $\delta^{17}O$ and the $\delta^{18}O$ values (Figure 3C). When using this approach, the $\Delta^{17}O$ results match those from the δ-calibration within analytical errors (±0.2‰, 1SD), except for the more ^{17}O-enriched ST-06, which is 1‰ more negative when using the Δ-calibration. Although no effect of evaporation during solutions handling has been found in our experiments, any water vapor loss

that could occur would affect the $\delta^{17}O$ and $\delta^{18}O$ measurements more than the $^{17}O_{excess}$, because in the first stages of the evaporation $^{17}O_{excess}$ changes less than the $\delta^{17}O$ and $\delta^{18}O$ values[9,11]. We suggest that the Δ-calibration can produce better long-term reproducibility for $\Delta^{17}O$, avoiding all analytical artefacts.

The accuracy of the IE-CRDS method was evaluated by analyzing the reference material IAEA-NO3 as an unknown against USGS-34 and USGS-35, using different values of β. We found that the measured $\Delta^{17}O$ value of IAEA-NO3 differs by less than 0.4‰ for $\beta \geq 0.007$ using the Δ-calibration compared with its accepted value (-0.2‰), with no improvement with further increased sample size (Figure 4A). For the $\delta^{18}O$ value in IAEA-NO3, the results obtained by IE-CRDS for $\beta \geq 0.007$ deviate by less than 1‰ on average from the accepted value (25.6‰), and similarly with no improvement with increased sample size (Figure 4B). These deviations in the $\Delta^{17}O$ and $\delta^{18}O$ values are within the 2SD analytical error of the method, demonstrating that IE-CRDS is accurate for $\Delta^{17}O$ determination in nitrate.

3.4. Sample size reduction

The general IE-CRDS method described above used 500 μL of acid solution that is equilibrated with at least ~118 μmol of NO_3^- (e.g. 10 mg of $NaNO_3$) to achieve a $\Delta^{17}O$ precision of ±0.2‰. Note that nitrate concentrations in unpolluted freshwater are normally less than 40 μmol/L[25-26], while concentrations lower than 20 μmol/L[22] and typical less than 5 μmol/L[34] can be found in snow and ice. This means that approximately 6 L of freshwater and up to 50 L of melted ice would be required for duplicate $\Delta^{17}O$ analyses of nitrate using the general IE-CRDS method. Considering that sample amount can be a limiting factor for some applications, we modified the IE-CRDS technique to reduce the amount of nitrate needed.

The practical lower volume limit of the $O\text{-}NO_3^-/O\text{-}H_2O$ equilibration experiments is determined by the volume of ~20 μL that the Picarro L2140i analyzer uses for the analysis of each sample (10 injections, 2 μL each). In practice, we reduced the solution volume to 50 μL (see section 2.2.2). This reduces the possibility of “bad injections” (low water concentration in the analyzer) for the last injection of each

sample that may occur when less than ~20 μL remains in the vial. This modification enables us to decrease the nitrate sample mass by one order of magnitude, to less than 9 μmol of NO_3^- keeping β constant (e.g. 0.0091). The experiments using this protocol of O-NO_3^-/O-H_2O isotope equilibration yielded $\Delta^{17}O$ results in perfect agreement with those from the general equilibration procedure, with acceptable precision (e.g. 0.5‰ for $\Delta^{17}O$ values and 3‰ for $\delta^{18}O$ values on average when 9 μmol of NO_3^- is used) and accurate values for the IAEA-NO3 reference material (calibrated values of 25.1‰ and -0.2‰ for $\delta^{18}O$ and $\Delta^{17}O$, respectively).

In practice, ~1 L of unpolluted freshwater (e.g. 20 μmol/L of NO_3^-) would be required to obtain enough nitrate for the duplicate analyses using the IE-CRDS method for small samples, while over 4L of melt ice/snow (e.g. 5 μmol/L of NO_3^-) would be needed. These sample volumes are considerably larger than those used by the bacterial denitrification methods[22,25,26,34], but similar to those for the TD-IRMS methods[1] (Table 1).

3.5. $\Delta^{17}O$ values in natural nitrates

The three natural samples analyzed using the IE-CRDS method yielded $\Delta^{17}O$ values ranging from 14.6‰ to 21.3‰, in good agreement with previous results of Atacama nitrates reported by Michalski et al[3] (Figure 6). The average precision of the duplicated measurements was better than 1.1‰. Our method is slightly less precise for natural samples than other methods (e.g. the TD-IRMS method[32]) but the reproducibility is considerably better than that of other laser-based procedures (e.g. the Cd-azide method using laser spectroscopy[42]). The precision for $\delta^{18}O$ values was better than 3.2‰ and was 2.1‰ on average. The $\delta^{18}O$ values obtained by the IE-CRDS method ranged from 42.4‰ to 55.9‰ and were identical to those obtained by TC/EA-IRMS, within the 2SD errors of the two methods. Our results for the Atacama nitrate samples plot in the same $\delta^{18}O$-$\Delta^{17}O$ space as the data set reported previously[3], suggesting good agreement between the IE-CRDS method and the traditional TD-IRMS method. We suspect that the slightly lower precision for $\delta^{18}O$ and $\Delta^{17}O$ values obtained for natural samples using the IE-CRDS than that for synthetic nitrates is because of small amounts of impurities that remained after sample preparation or because of incomplete O-NO_3^-/O-H_2O isotope equilibration

due to the presence of trace AgCl or Ag_2O in the experiments when using $AgNO_3$ as the starting material. The presence of impurities (i.e. other salts and organic compounds) may affect the accuracy of the IE-CRDS method for natural samples, resulting in systematically lower $\Delta^{17}O$ values than expected. This could be improved by using additional stages of purification by column resins or a different purification method.

It is worth mentioning that the NO_3^- concentrations in soils from dry deserts such as Atacama can exceed 15% in weight, so small soil samples (of the order of grams) are sufficient to obtain enough nitrate for the IE-CRDS analyses. Much smaller nitrate amounts are found in soils of wet areas, freshwaters[25-26] and ice/snow[22,34]; thus, larger sample amount would be required. This poses the largest disadvantage of the IE-CRDS method compared with other methods that can quantify considerably smaller nitrate amounts (e.g. denitrification-IRMS methods).

4. Conclusions

The IE-CRDS method presented here for determining $\Delta^{17}O$ values in solid NO_3^- provides a new application for CRDS technology previously used for triple oxygen analysis of waters. The reproducibility (1SD) can be better than 1.1‰ for $\Delta^{17}O$ value when nitrates from soils or salt mixtures are purified using a resin column and ~120 μmol of NO_3^- is used. This $\Delta^{17}O$ precision is not better than that obtained by other methods, but may be improved by increasing the number of replicates or sample size when possible. The precision for $\delta^{18}O$ values is generally better than 3‰, which can be suitable for some applications. Smaller sample amounts (down to ~9 μmol of NO_3^-) can be used if the volume of acid solution is reduced, producing similar reproducibility for $\delta^{18}O$ and $\Delta^{17}O$ values. This sample size is similar to that used by most TD-IRMS methods, but considerably larger than the NO_3^- amount required by the bacteria dentification-IRMS methods and the Cd-azide-laser spectroscopy method. Sample size can therefore limit the use of the IE-CRDS method for some applications.

The IE-CRDS method requires NO_3^- purification by resin column and reprecipitation as $AgNO_3$, representing the most time-consuming and costly step of the procedure. The TD-IRMS methods require the same sample purification protocol; however, the bacteria dentification-IRMS techniques and the Cd-azide-laser spectroscopy method can use solutions with complex matrices, reducing the preparation time and use of expensive reagents (e.g. Ag_2O). Importantly, the accuracy of the IE-CRDS method for natural samples relies on the purity of $AgNO_3$ obtained after purification; thus, impurities (i.e. other salts and organic compounds) may result in systematically lower $\Delta^{17}O$ values than expected. Additional purification protocols may be needed when analyzing samples with complex matrices by the IE-CRDS method.

The sample throughput of the IE-CRDS is ~12 samples/day, limited by the run time of the CRDS analyzer. This is less than the yield of other methods, but the isotopic analysis is highly automated and utilizes a benchtop instrument that is less expensive to purchase, run, and maintain than IRMS instruments.

In summary, the IE-CRDS method is a new analytical tool for isotope laboratories using CRDS analyzers for triple oxygen isotopes analysis in waters and represents an alternative to the traditional IRMS-based methods for measuring $\Delta^{17}O$ values in NO_3^-. CRDS instruments are becoming increasingly popular because they are cheaper and more user-friendly than mass spectrometers. However, the characteristics of the samples subject to study (e.g. NO_3^- concentration) should be carefully considered when choosing a method for measuring $\Delta^{17}O$ values in NO_3^-.

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References

1. Mauersberger K, Erbacher B, Krankowsky D, Gunther J, Nickel R. Ozone isotope enrichment: isotopomer-specific rate coefficients. *Science.* 1999;283:370–372.
2. Michalski G, Scott Z, Kabiling M, Thiemens MH. First measurements and modeling of $\Delta^{17}O$ in atmospheric nitrate. *Geophys Res Lett.* 2003;30(16):1870.
3. Michalski G, Böhlke JK, Thiemens MH. Long term atmospheric deposition as the source of nitrate and other salts in the Atacama Desert, Chile: New evidence from mass-independent oxygen isotopic compositions. *Geochim Cosmochim Acta.* 2004;68:4023-4038.
4. Jenkins KA, Bao H. Multiple oxygen and sulfur isotope compositions of atmospheric sulfate in Baton Rouge, LA, USA: *Atmos Environ*. 2006;40:4528-4537.
5. Clayton RN, Onuma N, Mayeda TK. A classification of meteorites based on oxygen isotopes. *Earth Plant Sci Lett.* 1976;30(1):10-18.
6. Herwartz D, Pack A, Friedrichs B, Bischoff A. Identification of the giant impactor Theia in lunar rocks. *Science.* 2014;344:1146-1150.
7. Ali A, Jabeen I, Gregory D, Verish R, Banerjee NR. New triple oxygen isotope data of bulk and separated fractions from SNC meteorites: Evidence for mantle homogeneity of Mars. *Meteorit Planet Sci*. 2016;51(5):981-995.
8. Luz B, Barkan E. Variations of $^{17}O/^{16}O$ and $^{18}O/^{16}O$ in meteoric waters. *Geochim Cosmochim Acta.* 2010;74:6276-6286.
9. Surma J, Assonov S, Bolourchi MJ, Staubwasser M. Triple oxygen isotope signatures in evaporated water bodies from the Sistan Oasis, Iran. *Geophys Res Lett.* 2015;42:8456-8462.

10. Alexander B, Thiemens MH, Farquhar J, Kaufman AJ, Savarino J, Delmas RJ. East Antarctic ice core sulfur isotope measurements over a complete glacialinterglacial cycle. *J Geophys Res.* 2003;D24:181–187.

11. Gázquez F, Morellón M, Bauska T, et al. Triple oxygen and hydrogen isotopes of gypsum hydration water for quantitative paleo-humidity reconstruction. *Earth Planet Sci Lett.* 2018;481C:177-188.

12. Landais A, Barkan E, Yakir D, Luz B. The triple isotopic composition of oxygen in leaf water. *Geochim Cosmochim Acta.* 2006;70:4105-4115.

13. Bao H, Lyons JR, Zhou C. Triple oxygen isotope evidence for elevated CO_2 levels after a Neoproterozoic glaciation. *Nature.* 2008;453:504–506.

14. Cao XB, Bao HM. Dynamic model constraints on oxygen-17 depletion in atmospheric O_2 after a snowball Earth. *Proc Natl Acad Sci.* 2013;110:14546-14550.

15. Böhlke JK, Sturchio NC, Gu B, et al. Perchlorate isotope forensics. *Anal Chem.* 2005;77:7838-7842.

16 Michalski G, Earman S, Dahman C, Hershey RL, Mihevc T. Multiple isotope forensics of nitrate in a wild horse poisoning incident. *Forensic Sci Int.* 2011;198(1-3):103-109.

17. Bao H, Cao X, Hayles JA. Triple Oxygen Isotopes: Fundamental Relationships and Applications. Annu Rev *Earth Planet Sci.* 2016;44:463-492.

18. Thiemens MH. History and applications of mass-independent isotope effects. Annu Rev *Earth Planet Sci.* 2006;34:217-262.

19. Gázquez F, Calaforra JM, Evans NP, Hodell DA. Using stable isotopes ($\delta^{17}O$, $\delta^{18}O$ and δD) of gypsum hydration water to ascertain the role of water condensation in the formation of subaerial gypsum speleothems. *Chem Geol.* 2017;452:34-46.

20. Levin NE, Raub TD, Dauphas N, Eiler JM. Triple oxygen isotope variations in sedimentary rocks. *Geochim Cosmochim Acta.* 2014;139:173-189.

21. Passey BH., Hu H, Ji H, et al. Triple oxygen isotopes in biogenic and sedimentary carbonates. *Geochim Cosmochim Acta.* 2014; 141:1-25.

22. Buffen AM., Hastings MG, Thompson LG, Mosley-Thompson E. Investigating the preservation of nitrate isotopic composition in a tropical ice core from the Quelccaya Ice Cap, Peru, *J Geophys Res Atmos*. 2014;119:2674-2697.

23. Fang Y, Koba K, Makabe A, et al. Microbial denitrification dominates nitrate losses from forest ecosystems. *Proc Natl Acad Sci.* 2015;112 (5):1470-1474.

24. Nakagawa F, Suzuki A, Daita S, Ohyama T, Komatsu DD, Tsunogai U. Tracing atmospheric nitrate in groundwater using triple oxygen isotopes: evaluation based on bottled drinking water. *Biogeosci*. 2013;10:3547-3558.

25. Tsunogai U, Komatsu DD, Daita S, et al. Tracing the fate of atmospheric nitrate deposited onto a forest ecosystem in Eastern Asia using $\Delta^{17}O$. *Atmos Chem Phys.* 2010;10:1809-1820.

26. Tsunogai U, Daita S, Komatsu DD, Nakagawa F, Tanaka A. Quantifying nitrate dynamics in an oligotrophic lake using $\Delta^{17}O$. *Biogeosci*. 2011;8:687-702.

27. Barkan E, Luz B. High precision measurements of $^{17}O/^{16}O$ and $^{18}O/^{16}O$ ratios in H_2O, *Rapid Commun Mass Spectrom.* 2015;19:3737–3742.

28. Schoenemann SW, Schauer AJ, Steig EJ. Measurement of SLAP and GISP $\delta^{17}O$ and proposed VSMOW-SLAP normalization for ^{17}O-excess. *Rapid Commun Mass Spec.* 2013;27:582–590.

29. Assonov SS, Brenninkmeijer CAM. Reporting small $\Delta^{17}O$ values: existing definitions and concepts. *Rapid Commun Mass Spectrom.* 2015;19:627–636.

30. Michalski G, Bockheim, JG, Kendall C, Thiemens MH. Isotopic composition of Antarctic Dry Valley nitrate: Implications for NOy sources and cycling in Antarctica. *Geophys Res Lett.* 2005;32(13):L13817.

31. Cliff SS, Thiemens MH. The $^{18}O/^{16}O$ and $^{17}O/^{16}O$ ratios in atmospheric nitrous oxide: A mass-independent anomaly. *Science.* 1997;278:1774-1776.

32. Michalski GJ, Savarino JK, Bohlke, Thiemens, M. Determination of the total oxygen isotopic composition of nitrate and the calibration of a $\Delta^{17}O$ nitrate reference material. *Anal Chem.* 2002;74:4989-4993.

33. Kaiser JM, Hastings G, Houlton BZ, Röckmann T, Sigman DM. Triple oxygen isotope analysis of nitrate using the denitrifier method and thermal decomposition of NO. *Anal Chem.* 2007;79:599-607.

34. Morin S, Savarino J, Frey M, et al. Tracing the Origin and Fate of NOx in the Arctic Atmosphere Using Stable Isotopes in Nitrate. *Science*. 2008;322:730-732.

35. Kunasek SA, Alexander B, Steig EJ, Hastings MG, Gleason DJ, Jarvis JC. Measurements and modelling of ^{17}O of nitrate in snowpits from Summit, Greenland, *J Geophys Res.* 2008;113:D24302.

36. Schauer AJ, Kunasek SA, Sofen ED, et al. Oxygen isotope exchange with quartz during pyrolysis of silver sulfate and silver nitrate. *Rapid Commun Mass Spectrom.* 2012;26:2151-2157.

37. Komatsu DD, Ishimura T, Nakagawa F, Tsunogai U. Determination of the $^{15}N/^{14}N$, $^{17}O/^{16}O$, and $^{18}O/^{16}O$ ratios of nitrous oxide by using continuous-flow isotope-ratio mass spectrometry. *Rapid Commun Mass Spectrom.* 2008;22:1587-1596.

38. McIlvin MR, Altabet MA. Chemical conversion of nitrate and nitrite to nitrous Oxide for nitrogen and oxygen isotopic analysis in freshwater and seawater. *Anal Chem.* 2005;77:5589–5595.

39. Tsunogai U, Kido T, Hirota A, Ohkubo SB, Komatsu DD, Nakagawa F. Sensitive determinations of stable nitrogen isotopic composition of organic nitrogen through chemical conversion into N_2O. *Rapid Commun Mass Spectrom*. 2008;22:345-354.

40. Dyckmans J, Lewicka-Szczebak D, Szwec L, Langel R, Well R. Comparison of methods to determine triple oxygen isotope composition of N. *Rapid Commun Mass Spectrom.* 2015;29:1991-1996.

41. Hattori S, Savarino J, Kamezaki K, et al. Automated system measuring triple oxygen and nitrogen isotope ratios in nitrate using the bacterial method and N_2O decomposition. *Rapid Commun Mass Spectrom*. 2016;30:2635-2644.

42. Wassennar WI, Douence C, Altabet MA, Aggarwal PK. N and O isotope ($\delta^{15}N\alpha$, $\delta^{15}N\beta$, $\delta^{18}O$, $\delta^{17}O$) analyses of dissolved NO_3^- and NO_2^- by the Cd-azide reduction method and N_2O laser spectrometry. *Rapid Commun Mass Spectrom.* 2018;32(3):184-194.

43. Berman ESF, Levin NE, Landais A, Li SN, Owano T. Measurement of $\delta^{18}O$, $\delta^{17}O$, and ^{17}O-excess in water by off-axis integrated cavity output spectroscopy and isotope ratio mass spectrometry. *Anal Chem.* 2013;85:10392-10398.

44. Steig EJ, Gkinis V, Schauer AJ, et al. Calibrated high-precision ^{17}O-excess measurements using laser-current tuned cavity ring-down spectroscopy. *Atmos Meas Tech.* 2014;7:2421-2435.

45. Kaseke KF, Wang L, Seely MK. Nonrainfall water origins and formation mechanisms. *Sci Adv.* 2017;3(3):e1603131.

46. Gázquez F, Mather I, Rolfe J, et al. Simultaneous analysis of $^{17}O/^{16}O$, $^{18}O/^{16}O$ and $^{2}H/^{1}H$ of gypsum hydration water by cavity ringdown laser spectroscopy. *Rapid Commun Mass Spectrom.* 2015;21:1997-2006.

47. Affolter S, Hauselmann AD, Fleitmann D, Hauselmann P, Leuenberger M. Triple isotope (δD, $\delta^{17}O$, $\delta^{18}O$) study on precipitation, drip water and speleothem fluid

inclusions for a Western Central European cave (NW Switzerland). *Quat Sci Rev*. 2015;127:73-89.

48. Gázquez F, Claire MW. Triple oxygen isotope analysis of nitrate using cavity ringdown laser spectroscopy. Geophysical Research Abstracts. EGU general Assembly. Vienna. 2018.

49. Kaneko M, Paulso SR. The rate of oxygen isotope exchange between nitrate and water. *Geochim Cosmochim Acta.* 2013;118:148-156.

50. Mook WG. *Environmental Isotopes in the Hydrological Cycle: Principles and Applications, Vol. I: Introduction - Theory, Methods, Review*. UNESCO, IAEA, Paris; 2000.

51. Bohlke JK, Mroczkowski SJ, Coplen TB. Oxygen isotopes in nitrate: new reference materials for ^{18}O:^{17}O:^{16}O measurements and observations on nitrate–water equilibration. *Rapid Commun Mass Spectrom.* 2003;17(16):1835-1846.

52. Silva SR, Kendall C, Wilkison DH, Ziegler AC, Chang CCY, Avanzino RJ. A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios. *J Hydrol.* 2000;228:22-36.

Table 1. Overview of analytical methods for triple oxygen isotopes measurements of nitrates

Method	Brief method description	Isotope measurements	NO_3^- amount	$\Delta^{17}O$ reproducibility (1SD)	Main advantages	Main disadvantages
Michalski et al[32]	Thermal decomposition (TD) of Ag_2NO_3 to O_2 in a quartz tube at 520°C.	O_2 by IRMS	6-40 μmol	0.2‰	Does not use hazardous reagents	NO_3^- purification required Relatively large NO_3^- samples Relatively low sample throughput
Kaiser et al[33]	Bacterial denitrification of NO_3^- to N_2O, followed by conversion of N_2O to O_2 in a gold tube at 800°C.	O_2 by IRMS	~50 nmol	0.5‰	Small NO_3^- samples Does not use hazardous reagents	Gold tube required
Kunasek et al[35] after Michalski et al[32]	Thermal decomposition (TC/EA) of Ag_2NO_3 to O_2 in a quartz tube at 550°C.	O_2 by IRMS	4-10 μmol	0.4‰	High sample throughput Does not use hazardous reagents	NO_3^- purification required Relatively large NO_3^- samples
Komatsu et al[37] after Tsunogai et al[39]	Chemical conversion of NO_3^- to N_2O using spongy cadmium.	N_2O by IRMS	~150 nmol	0.3‰	High sample throughput Small NO_3^- samples Provides $\delta^{15}N$ values	Potential interferences produced by dissolved NO_2^-
Morin et al[34] after Kaiser et al[33]	Automatization of the bacterial denitrification of NO_3^- to N_2O, followed by conversion of N_2O to O_2 in a gold tube at 800°C.	O_2 by IRMS	100 nmol	0.4‰	High sample throughput Small NO_3^- samples Provides $\delta^{15}N$ values	Gold tube required
Schauer et al[36] after Michalski et al[32] and Kunasek et al[35]	Thermal decomposition (TC/EA) of Ag_2NO_3 to O_2 in a quartz tube at 585°C using correction for quartz-O_2 isotope exchange.	O_2 by IRMS	>4 μmol	0.2‰	High sample throughput Does not use hazardous reagents	NO_3^- purification required Relatively large NO_3^- samples
Dyckmans et al[40] after Kaiser et al[33], and later automatation by Hattori et al[41]	Bacterial denitrification of NO_3^- to N_2O, followed by conversion of N_2O to O_2 by microwave induced plasma in a corundum tube.	O_2 by IRMS	~100 nmol	0.3‰	High sample throughput, automated protocol using small NO_3^- amounts Provides $\delta^{15}N$ values No need for NO_3^- purification	Very sensitive to sample amount
Wassenaar et al[42]	Conversion of dissolved NO_3^- to N_2O using the Cd-azide method and headspace N_2O by laser spectrometry.	N_2O by laser spectroscopy	~0.7 μmol NO_3^-	<9.1‰	Provides $\delta^{14}N$ and $\delta^{15}N^{\alpha}$ values Less expensive analytical instrument No need for NO_3^- purification	Uses hazardous reagents Highly imprecise for $\Delta^{17}O$ Potential interferences produced by dissolved NO_2^-.
This study	O-NO_3/O-H_2O isotopic equilibration in acid conditions at 80°C.	H_2O by laser spectroscopy	>9 μmol NO_3^-	0.2 to 1.1‰	Less expensive and user-friendly analytical instrument Does not use hazardous reagents	NO_3^- purification required Relatively large NO_3^- samples Relatively low sample throughput

Table 2. Calibrated triple oxygen isotope composition of synthetic and natural nitrates (soils and salt mixtures) analyzed by the IE-CRDS method proposed here and by the classics TD-IRMS and TC/EA-IRMS methods. $\Delta^{17}O$ has been calculated using Equation 1. The IE-CRDS results are the averaged values of experiments with $\beta \geq 0.007$. [See section 3.3. for definition of δ-calibration and Δ-calibration.]

		IE-CRDS									TD-IRMS	TC-EA	
							δ-calibration		Δ-calibration				
Sample	**Type**	**n**	**$\delta^{17}O$**	**1SD**	**$\delta^{18}O$**	**1SD**	**$\Delta^{17}O$**	**1SD**	**$\Delta^{17}O$**	**1SD**	**$\Delta^{17}O^{\#}$**	**$\delta^{18}O$**	**1SD**
ST-01	synthetic	14	48.5	1.1	46.1	1.1	23.6	0.6	23.5	0.5	23.2	47.2	0.8
ST-02	synthetic	14	18.6	0.9	20.3	1.7	7.9	0.4	7.8	0.3	7.4	19.0	0.9
ST-03	synthetic	14	13.2	1.8	26.4	2.8	-0.6	0.4	-0.5	0.6	-0.1	26.3	0.7
ST-05	synthetic	14	7.1	0.9	10.3	2.0	1.8	0.6	1.8	0.6	2.4	10.1	0.3
ST-06	synthetic	14	97.0	2.3	86.8	2.0	48.9	1.8	49.4	1.9	45.4	88.4	0.6
IAEA-NO3	synthetic	10	12.8	0.8	25.4	1.1	-0.5	0.4	-0.2	0.3	-	-	-
AT-12 PONR 90	natural	1	37.5	-	42.4	-	14.6	-	14.9	-	-	46.8	0.5
S-2	natural	2	52.1	1.7	55.9	3.2	21.3	0.0	21.1	0.0	-	53.3	0.5
S-6	natural	2	44.6	1.8	52.5	1.1	16.0	1.1	16.6	1.1	-	52.1	0.8

#The reproducibility of the $\Delta^{17}O$ value by the TD-IRMS method is better than 0.2‰.

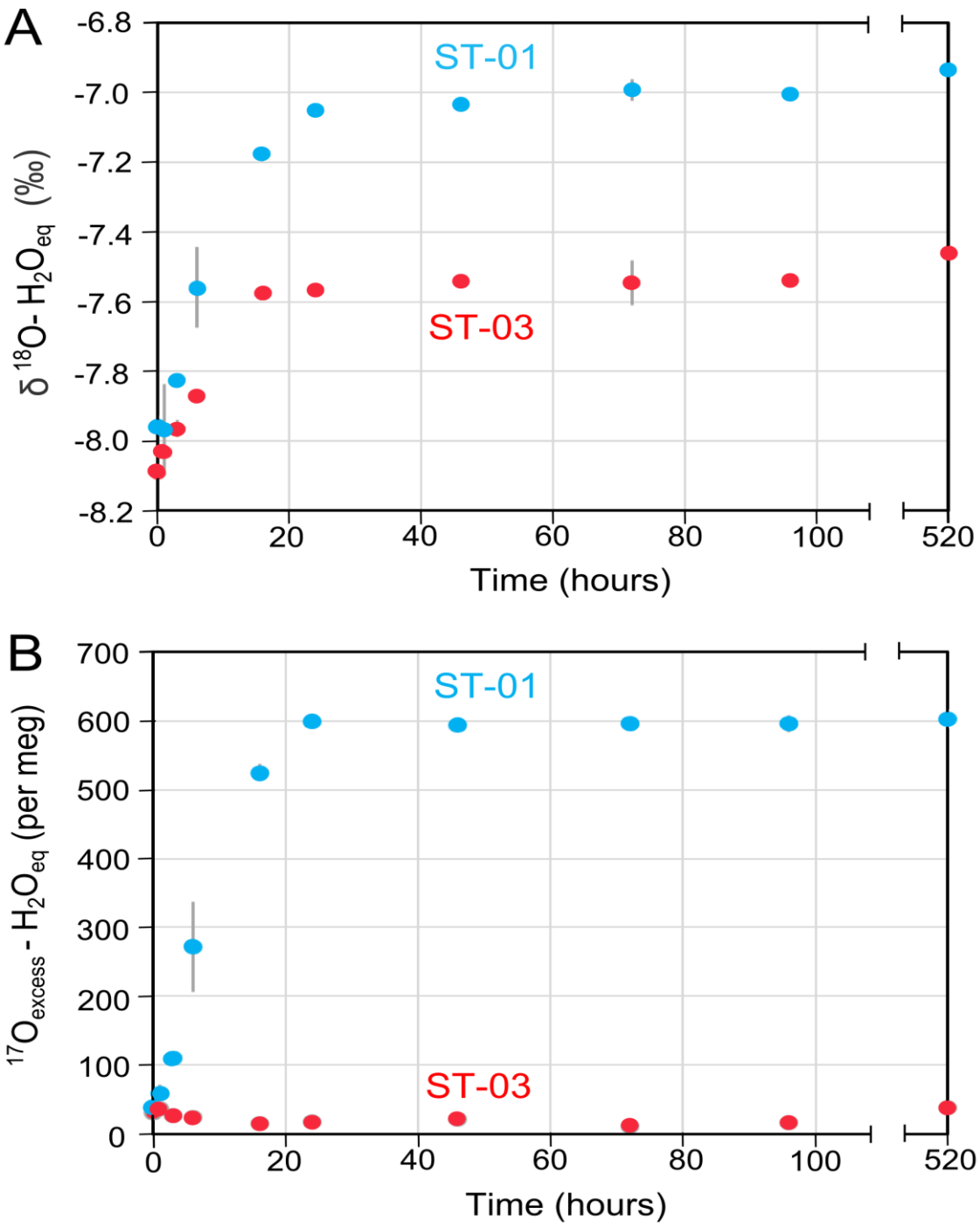

Figure 1. Triple oxygen isotope composition of water (ST-HCl) after O-NO_3^-/O-H_2O isotope equilibration with β of 0.0232±0.0001, at 80°C and pH=0.1 over 21 days. Experiments were performed using nitrate internal standards ST-01 (initial δ^{18}O-NO_3^- =47.2±0.8‰ and Δ^{17}O=23.2‰) and ST-03 (initial δ^{18}O-NO_3^-=26.3±0.7‰ and Δ^{17}O=-0.1‰). Experimental conditions reach isotopic equilibrium after 24 hours. The error bars, derived from duplicate analyses, are generally smaller than the symbols.

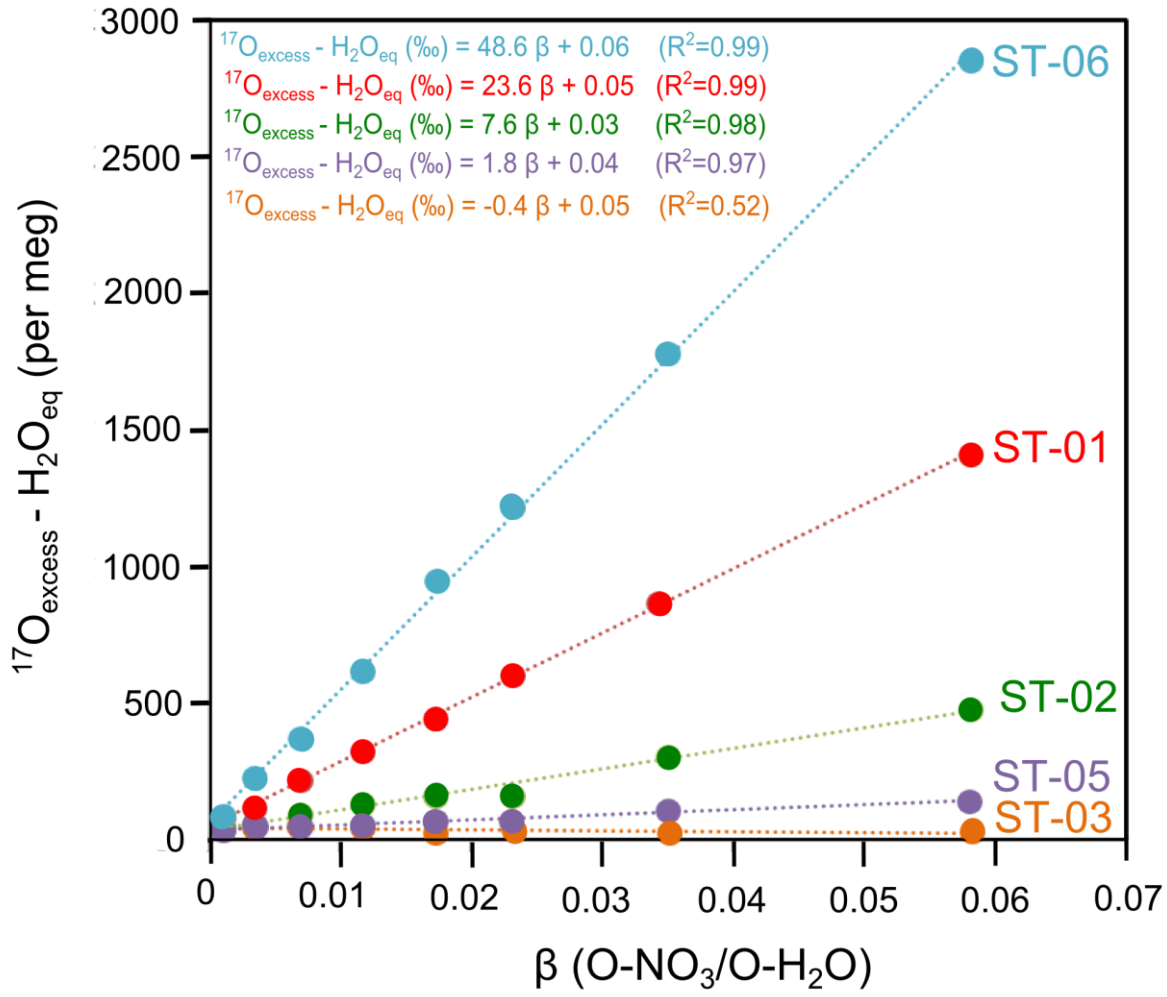

Figure 2. Triple oxygen isotope anomaly ($^{17}O_{excess}$) of water (ST-HCl) after complete equilibration with different amount of NO_3^- (β ranging from 0 to 0.0581) at 80°C and pH=0.1. The $\Delta^{17}O$ of nitrates ranges -0.1‰ to 45.4‰ (measured by TD-IRMS).

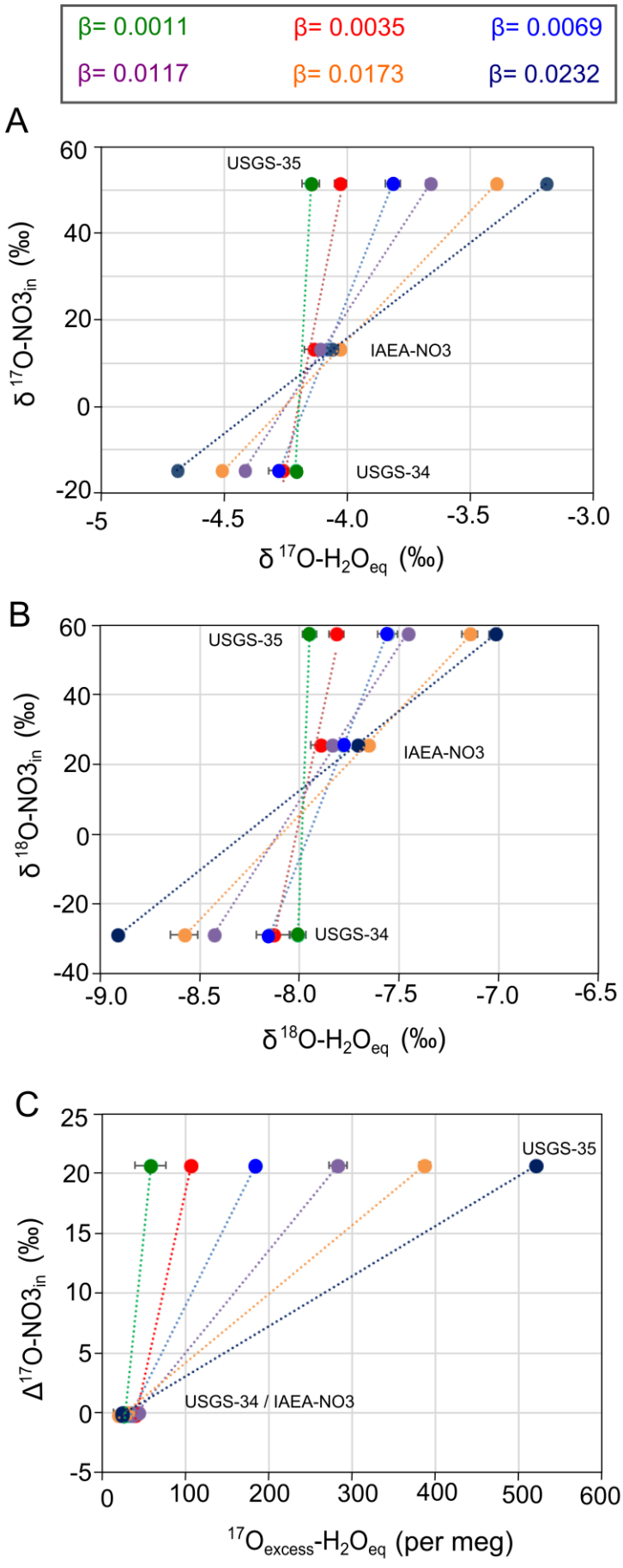

Figure 3. Calibration lines with international reference nitrate (USGS-34, USGS-35 and IAEA-NO3) and different O-NO_3^-/O-H_2O ratios (β). The isotope composition of the ST-HCl solution after equilibration (e.g. δ^{18}O-H_2O_{eq} value) with each reference nitrate has been plotted against the known initial isotopic compositions of the nitrate (e.g. δ^{18}O-$NO_{3\ in}^-$ value).

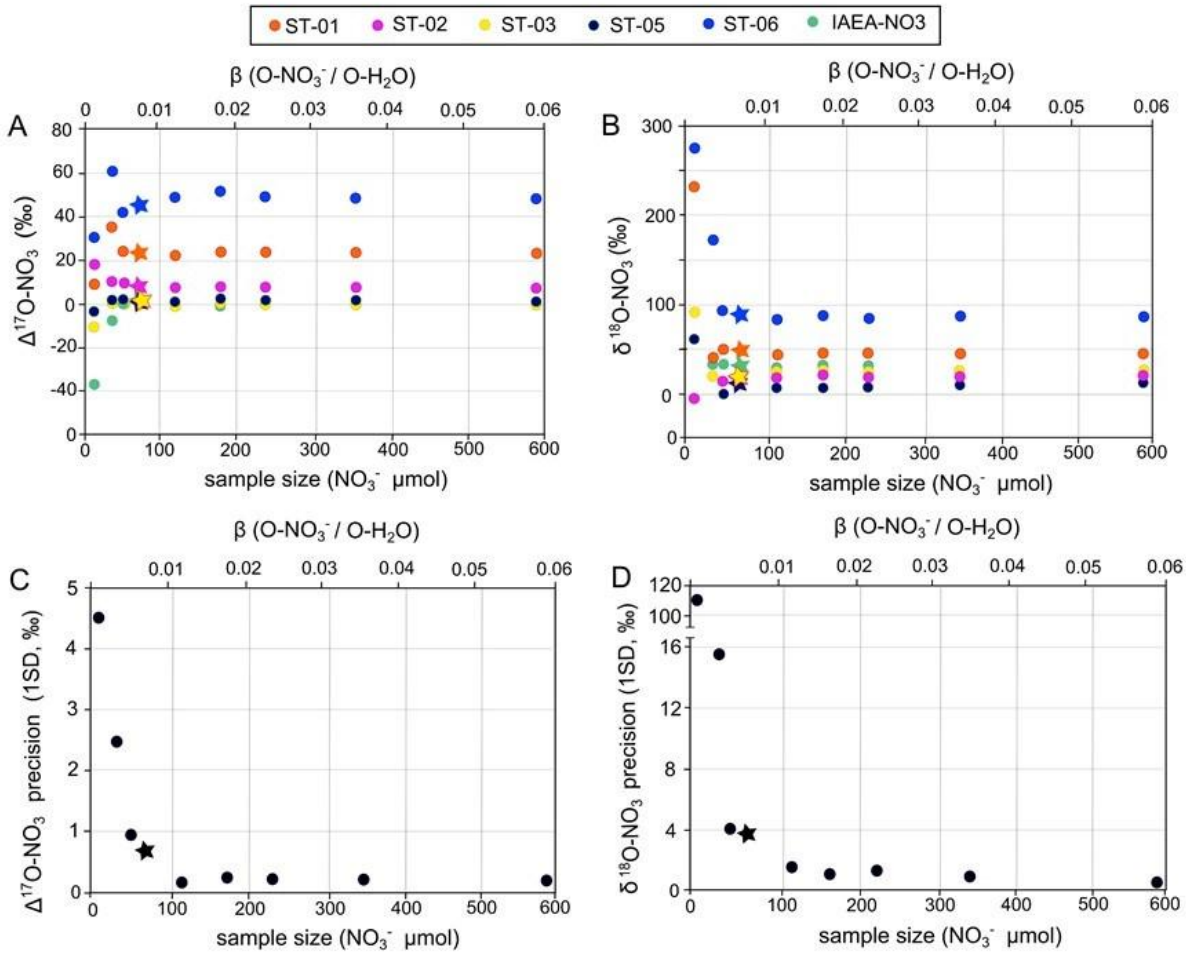

Figure 4. Triple oxygen isotope composition of synthetic nitrates determined by the IE-CRDS method. The $\Delta^{17}O$ values of nitrate were obtained by the Δ-calibration approach (see main text). The $\Delta^{17}O$ and $\delta^{18}O$ values stabilize for $\beta \geq 0.007$. Precision (1SD of duplicated analyses) improves with sample size (i.e. increased β) to 0.2‰ when $\beta \geq 0.0117$ is used, with no significant improvement above this value of β. Circles represent experiments that used the general $O\text{-}NO_3^-/O\text{-}H_2O$ isotope equilibration method (section 2.2.1) and stars denote experiments using microsamples (section 2.2.2). Note that sample sizes refer only to experiments that used 500 µL of acid solution. Experiments using smaller volumes (i.e. microsample experiments in section 3.4) require smaller nitrate amounts to maintain equivalent values of β.

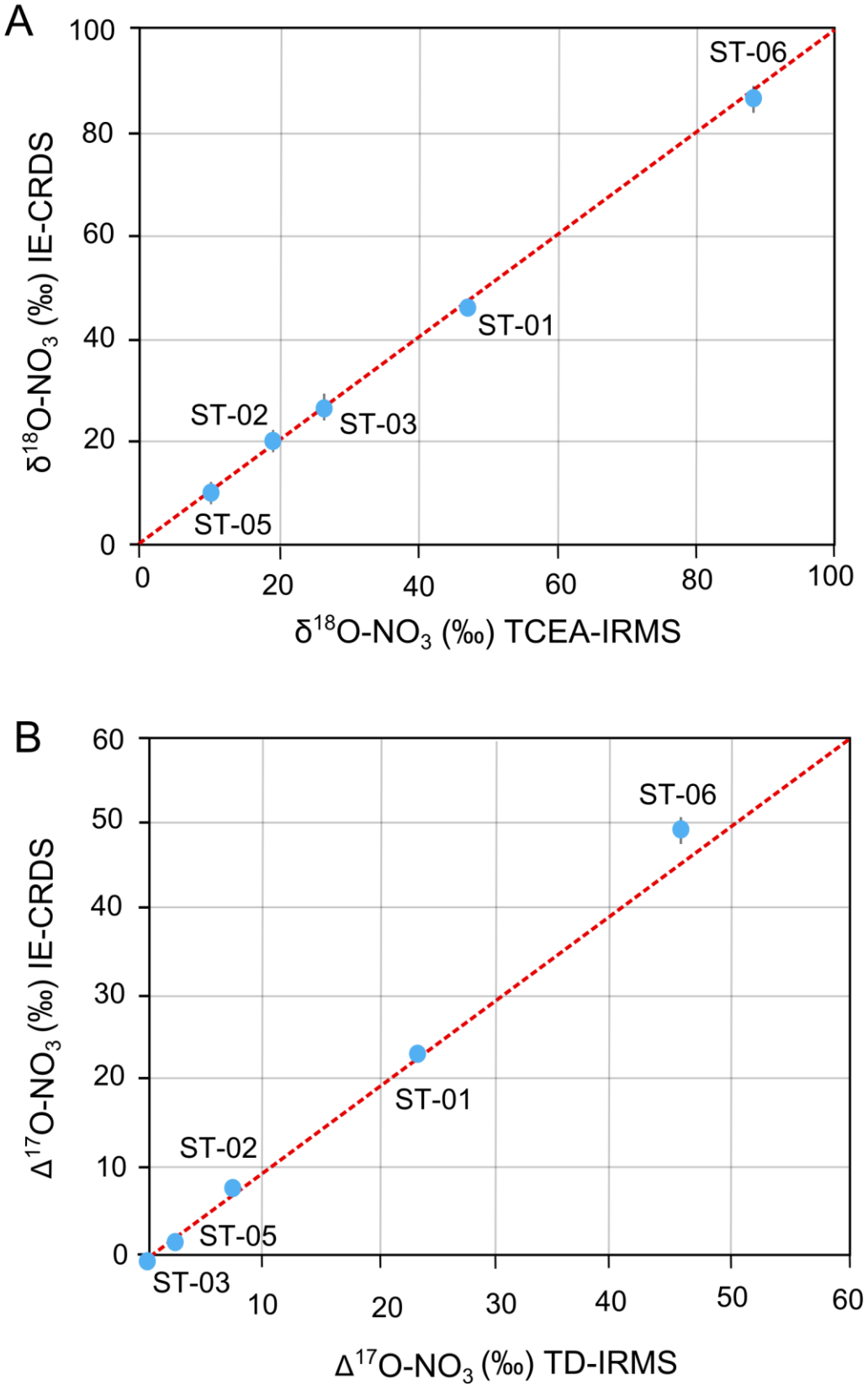

Figure 5. Cross-plot of $\delta^{18}O$ and $\Delta^{17}O$ values in synthetic nitrate samples analyzed by IE-CRDS compared with measurements of the same samples by TC/EA-IRMS

and TD-IRMS, respectively. The in-sample 1SD is given for the IE-CRDS (duplicate analysis of each sample) and TC/EA-IRMS (triplicate analysis of each sample) methods. The long-term 1SD errors of the TD-IRMS method (±0.2‰) is given for the results from this technique.

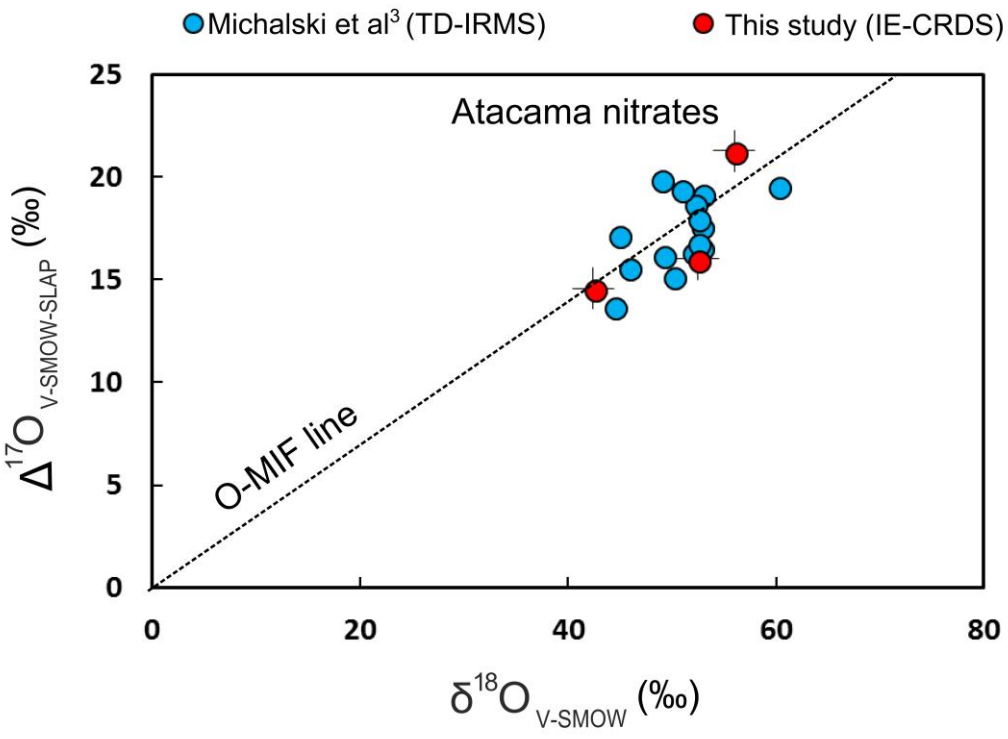

Figure 6. Triple oxygen isotope composition of natural nitrate samples from the Atacama Desert analyzed by the IE-CRDS method and previous results reported by Michalski et al[3].